

CLAIMS:

We claim:

1. A method for distinguishing bovines having a *CRH* gene polymorphism, comprising:

isolating a genomic DNA sample from a bovine;

amplifying a region of the bovine *CRH* gene using an oligonucleotide pair to form nucleic acid amplification products comprising amplified *CRH* gene polymorphism sequences;

detecting a polymorphism present in the *CRH* gene at position 22 of SEQ ID NO: 1;

analyzing the polymorphism, and

wherein the presence of a “G” residue is associated with the phenotypes of increased hot carcass weight, increased end-of-test rib-eye area and increased adjusted weaning weight, as compared to bovines with a “C” residue at position 22 of SEQ ID NO: 1.

2. The method of Claim 1 wherein the oligonucleotide pair comprises SEQ ID NO: 4 and SEQ ID NO: 5.
3. The method of Claim 2 wherein the polymorphism detected is a restriction fragment length polymorphism (RFLP).

4. The method of Claim 3 wherein the RFLP is the presence or absence of a *DdeI* restriction site at nucleotide 87 in a nucleic acid amplification product produced by amplification of the *CRH* gene using the oligonucleotide pair SEQ ID NO: 4 and SEQ ID NO: 5.
5. The method of Claim 1 further comprising the inclusion of a detectable moiety such that the amplification product comprises a labeled amplification product.
6. The method of Claim 5 wherein the detectable moiety is selected from the group consisting of fluorescent, bioluminescent, chemiluminescent, radioactive and colorigenic moieties.
7. The method of Claim 1 further comprising:
 - contacting the nucleic acid amplification products with a hybridization probe;
 - wherein the hybridization probes comprise at least one oligonucleotide labeled with a detectable moiety;
 - under suitable conditions permitting hybridization of the at least one oligonucleotide to the amplification product to form a hybridization complex;
 - and
 - wherein the presence of the detectable moiety in the hybridization complex indicates the presence of a *CRH* polymorphism.

8. The method of Claim 1 wherein the nucleic acid amplification product is produced by an amplification method selected from the group of polymerase chain reaction (PCR), strand displacement amplification (SDA), nucleic acid sequence based amplification (NASBA), rolling circle amplification, T7 polymerase mediated amplification, T3 polymerase mediated amplification and SP6 polymerase mediated amplification.

9. A method for distinguishing bovines having a *POMC* gene polymorphism, comprising:

isolating genomic DNA from a bovine;

amplifying a region of the bovine *POMC* gene using an oligonucleotide pair to form nucleic acid amplification sequences comprising amplified *POMC* gene polymorphism sequences;

detecting a polymorphism present in the *POMC* gene at position 254 of SEQ ID NO: 2;

analyzing the polymorphism, and

wherein the presence of a "T" residue is associated with the phenotypes of increased shipping weight and increased average daily gain as compared to bovines with a "C" residue at position 254 of SEQ ID NO: 2.

10. The method of Claim 9 wherein the oligonucleotide pair comprises SEQ ID NO: 6 and SEQ ID NO: 7.

11. The method of Claim 10 wherein the polymorphism detected is a restriction fragment length polymorphism (RFLP).
12. The method of Claim 11 wherein the RFLP is the presence or absence of a *BtsI* restriction site at nucleotide 157 in a nucleic acid amplification product produced by amplification of the *POMC* gene using the oligonucleotide pair SEQ ID NO: 6 and SEQ ID NO: 7.
13. The method of Claim 9 further comprising the inclusion of a detectable moiety such that the amplification product comprises a labeled amplification product.
14. The method of Claim 14 wherein the detectable moiety is selected from the group consisting of fluorescent, bioluminescent, chemiluminescent, radioactive and colorigenic moieties.
15. The method of Claim 9 further comprising:
 - contacting the nucleic acid amplification product with a hybridization probe;
 - wherein the hybridization probes comprise at least one oligonucleotide labeled with a detectable moiety;
 - under suitable conditions permitting hybridization of the at least one oligonucleotide to the amplification product to form a hybridization complex;
 - and

wherein the presence of the detectable moiety in the hybridization complex indicates the presence of a *POMC* polymorphism.

16. The method of Claim 9 wherein the nucleic acid amplification product is produced by an amplification method selected from the group of polymerase chain reaction (PCR), strand displacement amplification (SDA), nucleic acid sequence based amplification (NASBA), rolling circle amplification, T7 polymerase mediated amplification, T3 polymerase mediated amplification and SP6 polymerase mediated amplification.

17. A method for distinguishing bovines having a *MC4R* gene polymorphism, comprising:

isolating genomic DNA from a bovine;

amplifying a region of the bovine *MC4R* gene using an oligonucleotide pair to form nucleic acid amplification sequences comprising amplified *MC4R* gene polymorphism sequences;

detecting a polymorphism present in the *MC4R* gene at position 1069 of SEQ ID NO: 3;

analyzing the polymorphism, and

wherein the presence of a "G" residue is associated with the phenotype of increased hot carcass weight, as compared to bovines with a "C" residue at position 1069 of SEQ ID NO: 3.

18. The method of Claim 17 wherein the oligonucleotide pair comprises SEQ ID NO: 8 and SEQ ID NO: 9.
19. The method of Claim 17 wherein the polymorphism detected is a restriction fragment length polymorphism (RFLP).
20. The method of Claim 19 wherein the RFLP is the presence or absence of a *Tai*I restriction site at nucleotide 123 in a nucleic acid amplification product produced by amplification of the *MC4R* gene using the oligonucleotide pair SEQ ID NO: 8 and SEQ ID NO: 9.
21. The method of Claim 17 further comprising the inclusion of a detectable moiety such that the amplification product comprises a labeled amplification product.
22. The method of Claim 21 wherein the detectable moiety is selected from the group consisting of fluorescent, bioluminescent, chemiluminescent, radioactive and colorigenic moieties.
23. The method of Claim 17 further comprising:
- contacting the nucleic acid amplification product with a hybridization probe;
- wherein the hybridization probe comprises at least one oligonucleotide labeled with a detectable moiety;

under suitable conditions permitting hybridization of the at least one oligonucleotide to the amplification product to form a hybridization complex; and

wherein the presence of the detectable moiety in the hybridization complex indicates the presence of a *MC4R* polymorphism.

24. The method of Claim 17 wherein the nucleic acid amplification product is produced by an amplification method selected from the group of polymerase chain reaction (PCR), strand displacement amplification (SDA), nucleic acid sequence based amplification (NASBA), rolling circle amplification, T7 polymerase mediated amplification, T3 polymerase mediated amplification and SP6 polymerase mediated amplification.

25. An isolated and purified nucleic acid comprising a portion of the bovine *CRH* gene, further comprising a polymorphism at position 22 as defined by the positions in SEQ ID NO: 1, and in which there is a "C" residue at position 22. /

26. An isolated and purified nucleic acid comprising a portion of the bovine *POMC* gene, further comprising a polymorphism at position 254 as defined by the positions in SEQ ID NO: 2, and in which there is a "T" residue at position 254. /

27. A method of selecting individual livestock animals based on the knowledge of an animal's *CRH* genotype, comprising the steps of: /

determining the *CRH* alleles of an animal;

wherein the alleles of an animal will be one of “CC”, “CG”, or “GG” at position 22 of SEQ ID NO: 1; and

sorting animals into groups of like genotype; and

wherein a “CG” or “GG” genotype is associated with the desired phenotypes of increased hot carcass weight, increased end-of-test rib-eye area and increased adjusted weaning weight.

28. A method of selecting individual livestock animals based on the knowledge of an animal’s *POMC* genotype, comprising the steps of:

determining the *POMC* alleles of an animal;

wherein the alleles of an animal will be one of “CC”, “CT”, or “TT” at position 254 of SEQ ID NO: 2; and

wherein a “CT” or “TT” genotype is associated with the desired phenotypes of increased shipping weight, increased average daily gain and increased hot carcass weight.

29. A method of selecting individual livestock animals based on the knowledge of an animal’s *MC4R* genotype, comprising the steps of:

determining the *MC4R* alleles of an animal;

wherein the alleles of an animal will be one of “CC”, “CG”, or “GG” at position 1069 of SEQ ID NO: 3; and

wherein a “CG” or “GG” genotype is associated with increased hot carcass weight.

30. The method of Claim 29, wherein the only phenotype of interest is maximum increased hot carcass weight, and wherein an animal is first tested to determine the animal’s *MC4R* genotype;

and wherein, if the animal is homozygous for the “G” allele at the *MC4R* gene locus the animal is then tested to determine its *CRH* genotype, such that an animal that is homozygous for the “G” allele at the *CRH* gene locus will display the desired phenotype of maximum increased hot carcass weight.

31. The method of selecting individual livestock animals based on the knowledge of an animal’s *CRH* and *POMC* genotype, comprising the steps of:

determining the *CRH* and *POMC* alleles of an animal;

wherein the genotype of an animal will be one of “CC”, “CG” or “GG” at position 22 of SEQ ID NO: 1, and “CC”, “CT”, or “TT” at position 254 of SEQ ID NO: 2; and

wherein a “GG” genotype at the *CRH* gene and a “TT” genotype at the *POMC* gene (a “GG-TT” phenotype) is associated with the desired phenotypes of increased adjusted weaning weight, increased hot carcass weight, increased shipping weight, increased average daily gain and increased end-of-test rib-eye area, greater than that which would be obtained for animals homozygous for only one of the *CRH* and *POMC* loci.

32. A diagnostic kit for determining the *CRH* genotype of a bovine animal, the kit comprising:

oligonucleotide primers for amplifying a portion of the *CRH* gene;

the primers comprising a forward primer comprising at its 3' end sequence identical to at least 10 contiguous nucleotides within SEQ ID: 1;

a reverse primer comprising at its 3' end a nucleotide sequence fully complementary to at least 10 contiguous nucleotides with SEQ ID NO: 1;

and wherein the forward and reverse primers are from 10 to 30 nucleotides in length and in a PCR amplification reaction will produce a nucleic acid product amplification product containing a residue corresponding to position 2 of SEQ ID NO: 1.

33. The kit of Claim 32 wherein the primers comprise the oligonucleotides SEQ ID NO: 4 and SEQ ID NO: 5.

34. The kit of Claim 32 wherein the primers are labeled with a detectable moiety.

35. The kit of Claim 32 further comprising at least one oligonucleotide, labeled with a detectable moiety and suitable for use as a hybridization probe.

36. A diagnostic kit for determining the *POMC* genotype of a bovine animal, the kit comprising:

oligonucleotide primers for amplifying the *POMC* gene; /

the primers comprising a forward primer comprising at its 3' end sequence identical to at least 10 contiguous nucleotides within SEQ ID: 2;

a reverse primer comprising at its 3' end a nucleotide sequence fully complementary to at least 10 contiguous nucleotides with SEQ ID NO: 2;

at least one additional reagent selected from the group consisting of a lysing buffer for lysing cells contained in a sample, dNTP's, reaction buffer, an amplifying enzyme and a combination thereof.

and wherein the forward and reverse primers are from 10 to 30 nucleotides in length and in a PCR amplification reaction will produce a nucleic acid product amplification product containing a residue corresponding to position 254 of SEQ ID NO: 2.

37. The kit of Claim 36 wherein the primers comprise the oligonucleotides SEQ ID NO: 6 and SEQ ID NO: 7.

38. The kit of Claim 36 wherein the primers are labeled with a detectable moiety.

39. The kit of Claim 36 further comprising an oligonucleotide, labeled with a detectable moiety and suitable as a hybridization probe.

40. A kit for determining the *MC4R* genotype of an animal, the kit comprising:

oligonucleotide primers for amplifying the *MC4R* gene;

the primers comprising a forward primer comprising at its 3' end sequence identical to at least 10 contiguous nucleotides within SEQ ID: 3;

a reverse primer comprising at its 3' end a nucleotide sequence fully complementary to at least 10 contiguous nucleotides with SEQ ID NO: 3;

at least one additional reagent selected from the group consisting of a lysing buffer for lysing cells contained in a sample, dNTP's, reaction buffer, an amplifying enzyme and a combination thereof.

and wherein the forward and reverse primers are from 10 to 30 nucleotides in length and in a PCR amplification reaction will produce a nucleic acid product amplification product containing a residue corresponding to position 1069 of SEQ ID NO: 3.

41. The kit of Claim 40 wherein the primers comprise the oligonucleotides SEQ ID NO: 8 and SEQ ID NO: 9.
42. The kit of Claim 40 wherein the primers are labeled with a detectable moiety.
43. The kit of Claim 40 further comprising at least one oligonucleotide, labeled with a detectable moiety and suitable or use as a hybridization probe.

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